Measuring Tissue pO_2, Redox and pH by Magnetic Resonance

Weekend Educational Course on Molecular Imaging, ISMRM 2006, Seattle, WA

Natarajan Raghunand, Ph.D.

Dept. of Radiology, University of Arizona, Tucson, AZ 85724-5024

Tel.: (520) 626-6041; Fax: (520) 626-5051; raghunan@email.arizona.edu

Tissue energy metabolism can be characterized as the sequential oxidation of relatively reduced carbon sources with the transfer of reducing equivalents to NAD(P)+ or flavoproteins. These, in turn, use these electrons for energy production in mitochondria or for protection from reactive oxygen species, ROS. In either oxidative or fermentative metabolism, the endproducts of metabolism are acids, leading to low pH. This delicate balance of oxygen, redox ratios and pH can be upset in pathologies such as ischemia, renal failure, COPD, and cancer. Our understanding of the interrelationships between oxygen, redox and pH will be improved by the development of imaging methods to interrogate these parameters non-invasively. In this section, we will briefly describe current and novel approaches to the measurement of tissue oxygen, redox and pH.

MEASUREMENT OF TISSUE OXYGEN

Table 1 lists a number of oxygen measurement techniques that are in current development. The "gold standard" is pO_2 histography (usually with an Eppendorf microelectrode). Although it is invasive, this approach yields scalar and quantitative values for pO_2. If data are collected at multiple sites, a histogram of oxygen values within the organ of interest can be generated.

TABLE 1. Summary of Methods to Stratify Patients for Hypoxia-Directed Therapies.

Technique	Injection?	Measures	Resolution	Clinically
Invasive				
pO_2 histography	No	pO_2	0.5 mm	Approved
PET imaging				
^{18}F-Miso PET	Yes	hypoxia	2.0 mm	Phase III
^{18}F-AZA PET	Yes	hypoxia	2.0 mm	Phase II
^{18}F-EF5 PET	Yes	hypoxia	2.0 mm	Phase II
^{64}Cu-ATSM	Yes	hypoxia	3.0 mm	Phase III
MR imaging				
^{19}F-MRI	Yes	pO_2	2.0 mm	No
MRI BOLD R2*	No	Deoxy-Hb	0.2 mm	Phase II
Hi MW DCE MR	Yes	Permeability	0.2 mm	Phase II/III
EPRI Particulate	No	pO_2	1.0 mm	Phase II
EPRI Infusion	Yes	pO_2	1.0 mm	No
Histology				
HIF-1	No	Biological Hypoxia	1.0 µm	No
GLUT-1	No	HIF trans-activation	1.0 µm	Yes
CA 9	No	HIF trans-activation	1.0 µm	Yes
EF5	Yes	Chronic +	1.0 µm	Yes
Pimonidazole	Yes	Chronic +	1.0 µm	Yes
Serum Marker				
OPN in Serum	No	Chronic hypoxia?	N/A	Yes

It is notable that there are a number of Positron Emission Tomography (PET) tracers in development that are based either on reduction and trapping of 2-nitroimidazoles (miso, azamiso, EF5) or through intracellular enzymatic reduction and trapping of ^{64}Cu. Despite the fact that these produce images of poorer resolution compared to MRI, they are generally believed to be more quantitative and are being approved for human use. In contrast, MR techniques, despite their higher intrinsic resolution, are not yet validated to provide accurate, quantitative or relevant measures of tissue oxygenation. Both Electron Paramagnetic Resonance Imaging (EPRI) of particulates and ^{19}F MRI of hexafluorobenzene require implantation of reporters. Although measurements can be carried out longitudinally thereafter with these approaches, these are somewhat more invasive than other measures. Dynamic Contrast-enhanced MRI (DCE-MRI) of high molecular weight contrast reagents is sensitive to vessel permeability and this can be a downstream sequela of biological hypoxia. Although this is potentially promising as a biomarker for hypoxia, it is early in development. EPR imaging of infused contrast reagents is also early in development, yet holds promise for quantitative measures of pO_2 non-invasively. The following section will briefly discuss BOLD MRI in more detail because of its non-invasive nature and its wide applicability.

BLOOD OXYGEN LEVEL-DEPENDENT (BOLD) MRI

Deoxyhemoglobin (Hb●) in circulating red blood cells contains an unpaired electron. Thus it is paramagnetic, while oxygenated hemoglobin (HbO_2) is not. The paramagnetic effect causes a decrease in the apparent T2 (T2*) of nearby water protons through changes in the bulk magnetic susceptibility. BOLD contrast is generated from difference images between activated and resting tissues collected with T2* sensitive sequences, such as gradient-recalled echoes (GRE) [1]. Activation is commonly induced with hyperoxia, typically administered through carbogen (95%:5% O_2:CO_2) breathing. Quantification of the BOLD effect in response to carbogen is difficult because signal changes are affected by not only the tissue pO_2, but also the tissue pH, the hematocrit and blood flow [2]. Carbogen is used instead of 100% O_2 because it is believed that the increased CO_2 (hypercapnia) will block hyperoxic vasoconstriction. However, the effects of CO_2 on blood flow are complex, probably involving direct effects of CO_2 and indirect effects of the resulting lowered pH [3]. In Morris hepatomas, carbogen increased oxygenation and reduced interstitial pH, while no effects on blood flow were observed [4]. In GH3 prolactinomas, even 1% CO_2:99%O_2 caused a more significant drop in R2*, compared to 100% O_2 [5], indicating that even slight hypercapnia can have significant effects on vascular tone. This is important because the respiratory acidosis caused by 5% CO_2 causes significant patient discomfort, whereas 1% CO_2 is easily tolerable. Carbogen has been shown to induce reductions in tumor pH, which are likely due a direct effect of CO_2 hydration, rather than changes in perfusion [6, 7, 8]. Tissue acidification also decreases oxygen affinity through the Bohr effect, consequently increasing Hb● without changes in oxygen status. Patent vessels in tumors can have transiently low hematocrit leading to reductions in BOLD contrast, even under hypoxia [9]. Another shortcoming of the use of BOLD MRI to quantitatively measure blood oxygenation is that there can be a significant inflow of unsaturated spins during the long echo times (typically ≈ 60 ms), resulting in an underestimation of hypoxia. For this reason, changes in GRE contrast have been termed FLOOD (flow and oxygen dependent) contrast. With careful controls, the individual components can be deconvolved, showing that the major effect of carbogen on GRE contrast is a decrease in Hb● [2, 10, 11].

GRE-MRI has been used successfully to characterize tumor vascular dynamics by measuring relative responses to physiological challenges, such as hypercapnia, hyperoxia, and vasoactive drugs. In an important series of experiments, Neeman and colleagues have shown that dynamic changes in GRE images between normoxia, hypercapnia and carbogen can be used to

discriminate immature from mature vasculature. Mature vessels (i.e. those with pericytes or smooth muscle cells) respond to hypercapnia while immature vessels are unresponsive. This distinction has been used to measure the selective destruction of immature vessels in response to VEGF withdrawal [12]. Parallel studies using intravital microscopy showed that hypercapnia induced vasoconstriction in mature vessels, and the resulting drop in hematocrit caused a paradoxical drop in BOLD contrast, consistent with reduced Hb● [9, 12].

In addition to describing the microenvironment, these techniques can be applied to monitor the effects of improving therapy. Carbogen is being used to enhance chemotherapeutic efficacy, as in the case of 5-fluorouracil [13]. Carbogen causes delayed pharmacokinetics and a transient decrease in GRE signal intensity that is positively correlated with tumor size [7]. Although this was interpreted as an increase in oxygenation, this signal drop is likely caused by a transient reduction in hematocrit, since these treatments also resulted in a tumor size-correlated drop in tissue pH. In combination with radiotherapy, GRE-MRI images have been collected from patients in response to carbogen, showing that while normal tissues uniformly respond with decreased T2*, a significant fraction (11/28) of patient tumors are unresponsive [14, 15]. Work from a number of groups suggests that distinct components of the water resonance from each voxel respond differently to changes in oxygenation [16, 17]. Thus, subvoxelar, perhaps microscopic heterogeneity of changes in tumor oxygenation can be detected by high spectral and spatial resolution (HISS) multivoxel spectroscopy.

pH IMAGING

Historically, the extracellular pH (pHe) of tissues *in vivo* has been measured using microelectrodes or weak acid/weak base distributions [18, 19, 20]. These approaches have disadvantages in that they are either invasive or destructive. The measurement of pH using the chemical shift of endogenous inorganic phosphate by ^{31}P MRS represented the first time that this parameter was measured *in vivo* [21, 22, 23, 24]. Such measurements have greatly stimulated this field of inquiry.

Measurement of pH by ^{31}P MRS.

^{31}P measurements of human and animal pH using inorganic phosphate report values that are neutral-to-alkaline [25, 26, 27]. As these values were significantly higher than those observed with microelectrodes, particularly in tumors, it was reasoned that this technique was measuring intracellular pH (pHi) while microelectrodes measured primarily pHe [28]. This was confirmed through the use of an extracellular pH indicator, 3-aminopropylphosphonate (3-APP) which reported an acidic pHe and a neutral-to-alkaline pHi in tumor xenografts and neutral-to-alkaline pHe in normal tissues [29]. Since then, 3-APP has been a common tracer used during ^{31}P MRS of tissues, primarily tumors and muscle.

Measurement of pH by ^{1}H MRS.

^{1}H MRS offers significantly higher sensitivity compared to ^{31}P MRS, allowing for data to be collected in smaller voxels in less time. ^{1}H MRS approaches to measure pHe have relied on compounds such as imidazoles and aromatics that resonate far downfield of endogenous metabolites. The first such approaches to be used *in vivo* were based on imidazole-1-alkyl esters developed by Ballesteros and her colleagues [30, 31]. One of these, (+/-)2-imidazole-1-yl-3-ethoxycarbonylpropionic acid (IEPA), has been used in breast tumor xenografts to produce multivoxel pHe maps with resolution approaching 1 x 1 x 2 mm^3 [32]. These maps showed pHe values from 6.4-6.8, which were consistent with those measured with ^{31}P MRS of 3-APP. By combining MRSI of IEPA and vascular MRI using albumin-GdDTPA, Bhujwalla et al. [33] have demonstrated the feasibility of obtaining co-registered maps of vascular volume, permeability and extracellular pH. Studies correlating pHe to metabolites in a transplanted rat glioma model

showed negative correlations between N-acetyl aspartate and IEPA measured pHe or lactate, and surprisingly little correlation between lactate and pHe [34].

An alternative approach to measuring pH with single voxel MRS of imidazoles uses the chemical shift of free histidine H2 resonances. However, in most tissues except muscle, histidine levels are too low to observe. Oral supplementation of human subjects with approx. 0.4 g/kg increases histidine levels to 0.8 mM in brain, which can be observed in patients at 1.5 T in a reasonable timeframe. The (presumably intracellular) pH measured by this technique showed a brain pH of 6.96 [35].

Measurement of pH by ^{19}F MRS.

Compared to ^{31}P and ^{1}H, ^{19}F is advantageous in having both high sensitivity and no interference from endogenous resonances. Of course, a disadvantage of ^{19}F MR is a lack of other physiologic information, which must be interrogated with other nuclei. 20 years ago, Deutsch and her colleagues developed a series of fluorinated alanines for measurement of cell pH [36, 37]. Although they did not find utility *in vivo*, they clearly demonstrated the power of this approach. Currently, two different approaches are being used *in vivo*. Mason and colleagues are using fluorinated vitamin B6 derivatives (6-fluoropyridoxol and 6-fluoropyridoxamine) which distribute across plasmalemma and undergo a chemical shift, allowing simultaneous measurement of pHi and pHe [38, 39]. An alternative compound, 3-[N-(4-fluor-2-trifluoromethylphenyl)- sulphamoyl]-propionic acid (ZK-150471) has also been well-developed and yields pHe values consistent with those measured by ^{31}P MRS, but with higher precision in less time and free from compartmentation artifacts [40]. Similarly ZK-150471 has been used to measure tumor pHe upon hyperthermia or hydralazine treatment, showing significant decreases with both treatments [41]. Although fluorinated B6 and ZK-150471 have high sensitivity and a large pH-dependent chemical shift dispersion, they have not yet been used in an imaging protocol.

Measurement of pH by Contrast-Enhanced MRI.

pH-sensitive gadolinium complexes and gadolinium-containing pH-sensitive polyion complexes offer the possibility of imaging pH with a spatial resolution comparable to standard MRI. Aime and co-workers [42] have exploited the fact that at the magnetic fields typically employed in clinical scanners, the relaxivity of Gd(III) chelates is largely determined by the reorientational time of the molecule. They synthesized a complex, $(GdDO3ASQ)_{30}$-Orn_{114}, consisting of thirty Gd(III) chelates bound to a poly-ornithine chain. Protonation and hydration of free amino groups at acidic pH pushes them apart and increases the mobility of the paramagnetic moieties. On the other hand, progressive deprotonation of these groups with increasing pH renders the complex more rigid, slowing down the reorientational time experienced by individual chelates to values closer to that of the molecule as a whole. Thus, the longitudinal water proton relaxivity of the complex increases with increasing pH.

Sherry and co-workers have reported that the H-bonding network created by protonation of phosphonate side-arms of the GdDOTA-4AmP complex provides a catalytic pathway for exchange of the bound water protons with protons of bulk water, making the longitudinal water proton relaxivity of this molecule pH-sensitive [43]. The enhancement produced by such agents is dependent on both the local pH and concentration of the agent. Hence, one approach to determine pH using these compounds is to sequentially administer two contrast agents having identical tissue pharmacokinetics, with one being insensitive to tissue pH [44]. The distribution of the pH-insensitive agent can be used to predict the concentration of the pH-sensitive agent. Using GdDOTP as a pH-insensitive surrogate for GdDOTA-4AmP, it has been possible to compute pH images of kidneys and nearby tissues in mice [45].

Measurement of pH by Magnetization Transfer.
An interesting new approach to the measurement of tissue pH is to measure a pH-dependent chemical exchange dependent magnetization (saturation) transfer, CEST. This approach has its foundations in structural biology, wherein phase-cycling is used to determine amide-hydrogen exchange (e.g., [46]). Van Zijl and colleagues have developed methods to measure these exchange rates *in vivo*, by avoiding saturation of water protons during solvent suppression. Consequently, this generates a significant improvement of signal intensities from exchangeable hydrogens, many of which reside in the amide part of the spectrum, between 5-10 ppm. The intensities of these resonances are affected by pH-dependent exchange relayed saturation [47]. This approach, however, is difficult to quantify since the concentration of exchanging amides is not known. Furthermore, it is limited to MRS acquisitions. Balaban and colleagues have begun to develop probes with high exchange rates in order to directly transfer saturation to bulk water. Combining pH-sensitive and pH-insensitive exchanging species that resonate at different frequencies allows for a concentration-independent ratiometric determination of pH [46, 48, 49]. In these cases, the exchanging moieties can either be on the same compound, or on different compounds with similar biodistribution. A limiting factor in these studies are the high concentrations required (ca. 60 mM), and the direct magnetization transfer to water, as the exchanging hydrogens resonate within the water envelope. Optimal CEST contrast results when the exchange rate of the mobile protons with water approaches the separation (in Hz) between the chemical shift values of the two exchanging species: larger relative chemical shifts permit the exploitation of agents with faster water exchange rates. Aime and co-workers have recently reported the development of lanthanide-based paramagnetic complexes containing both highly shifted pH-insensitive and pH-sensitive exchangeable protons, allowing for a ratiometric determination of pH [50]. Sherry and co-workers have reported the development of a Europium-based CEST agent with a Eu-bound proton resonance which is 50 ppm downfield of the bulk water resonance [51]. Hence, there will be minimal direct transfer of magnetization to water, thereby further increasing the contrast. This compound has been further derivatized to provide a pH-dependent CEST effect [52].

MEASUREMENT OF REDOX BY MRI.
Tissue redox status is known to be important in several disease states, particularly pathological conditions involving hypoxia, including tumors, strokes and myocardial infarcts [53, 54]. EPR evidence that hypoxic regions in xenograft tumors are highly reducing has been uncovered in studies by the groups of Kuppusamy, Zweier and Gallez [55, 56, 57]. Emerging evidence indicates that a reducing extracellular microenvironment aids tumor cell survival and proliferation [58, 59, 60, 61]. Several drugs have been designed to target tumor cells in such hypoxic-reducing microenvironments [62, 63, 64]. Efforts are ongoing to create redox-sensitive contrast agents for MRI that would enable the non-invasive assessment of tissue redox. Aime and co-workers have proposed exploiting the redox-sensitive Mn(II)/Mn(III) transition for this purpose [65]. The Eu(II)/Eu(III) transition holds even greater potential in this regard, as the reduced Eu(II) state is isoelectronic with Gd(III) and is an excellent T1 relaxing agent, while the oxidized Eu(III) state is poorly relaxing [66].

We have synthesized thiol complexes of gadolinium designed to spontaneously form reversible covalent linkages with circulating plasma albumin at the Cys^{34} residue. The objective is to keep the gadolinium bound to albumin, and therefore intravascular, in oxidizing microenvironments. Reduction of the disulfide bond in reducing microenvironments would free the gadolinium complex, rendering it small enough to extravasate. Strategies for exploiting such redox-sensitive binding of contrast agents to albumin to image tumor redox will be discussed.

References

1. Robinson SP, Howe FA, Rodrigues LM, et al. Magnetic resonance imaging techniques for monitoring changes in tumor oxygenation and bloodflow. Semin Radiat Oncol 1998;8:197–207.
2. Howe FA, Robinson SP, Rodrigues LM, et al. Flow and oxygenation dependent (FLOOD) contrast MR imaging to monitor the response of rat tumors to carbogen breathing. Magn Reson Imaging 1999;17:1307-1318.
3. Dunn TJ, Braun RD, Rhemus WE et al. The effects of hyperoxic and hypercarbic gases on tumour blood flow. British Journal of Cancer 1999;80:117-126.
4. Stubbs M, Robinson SP, Rodrigues LM et al. The effects of host carbogen (95% oxygen/5% carbon dioxide) breathing on metabolic characteristics of Morris hepatoma 9618a. British Journal of Cancer 1998;78:1449-1456.
5. Robinson SP, Rodrigues LM, Howe FA et al. Effects of different levels of hypercapnic hyperoxia on tumour R(2)* and arterial blood gases. Magnetic Resonance Imaging 2001;19:161-166.
6. Howe FA, Robinson SP, Griffiths JR. Modification of tumour perfusion and oxygenation monitored by gradient recalled echo MRI and 31P MRS. NMR in Biomedicine 1996;9:208-216.
7. McSheehy PM, Robinson SP, Ojugo AS et al. Carbogen breathing increases 5-fluorouracil uptake and cytotoxicity in hypoxic murine RIF-1 tumors: a magnetic resonance study in vivo. Cancer Res 1998;58:1185-1194.
8. Robinson SP, Rodrigues LM, Griffiths JR et al. Response of hepatoma 9618a and normal liver to host carbogen and carbon monoxide breathing. Neoplasia 1999;1:537-543.
9. Neeman M, Dafni H, Bukhari O et al. In vivo BOLD contrast MRI mapping of subcutaneous vascular function and maturation: validation by intravital microscopy. Magnetic Resonance in Medicine 2001;45:887-898.
10. Robinson SP, Collingridge DR, Howe FA et al. Tumour response to hypercapnia and hyperoxia monitored by FLOOD magnetic resonance imaging. NMR in Biomedicine 1999;12:98-106.
11. Howe FA, Robinson SP, McIntyre DJ et al. Issues in flow and oxygenation dependent contrast (FLOOD) imaging of tumours. NMR in Biomedicine 2001;14:497-506.
12. Abramovitch R, Dafni H, Smouhas E et al. In vivo prediction of vascular susceptibility to vascular endothelial growth factor withdrawal: magnetic resonance imaging of C6 rat glioma in nude mice. Cancer Res 1999;59:5012-5016.
13. Griffiths JR, McIntyre DJ, Howe FA et al. Issues of normal tissue toxicity in patient and animal studies--effect of carbogen breathing in rats after 5-fluorouracil treatment. Acta Oncologica 2001;40:609-614.
14. Griffiths JR, Taylor NJ, Howe FA et al. The response of human tumors to carbogen breathing, monitored by Gradient-Recalled Echo Magnetic Resonance Imaging. Int J Rad Oncol Biol Phys 1997;39:697-701.
15. Taylor NJ, Baddeley H, Goodchild KA et al. BOLD MRI of human tumor oxygenation during carbogen breathing. Journal of Magnetic Resonance Imaging 2001;14:156-163.
16. Al Hallaq HA, Fan X, Zamora M et al. Spectrally inhomogeneous BOLD contrast changes detected in rodent tumors with high spectral and spatial resolution MRI. NMR in Biomedicine 2002;15:28-36.
17. Posse S, Wiese S, Gembris D et al. Enhancement of BOLD-contrast sensitivity by single-shot multi-echo functional MR imaging. Magnetic Resonance in Medicine 1999;42:87-97.
18. Wike-Hooley JL, Haveman J, Reinhold HS. The relevance of tumour pH to the treatment of malignant disease. Radiotherapy and Oncology 1984;2:343-366.
19. Volk T, Jahde E, Fortmeyer HP et al. pH in human tumour xenografts: effect of intravenous administration of glucose. Br.J.Cancer 1993;68:492-500.
20. Waddell WJ, Butler TC. Calculation of intracellular pH from the distribution of 5,5- dimethyl-2,4-oxazolidinedione (DMO). Application to skeletal muscle of the dog. J Clin Invest 1959;38:720-729.
21. Evelhoch JL, Sapareto SA, Jick DE et al. In vivo metabolic effects of hyperglycemia in murine radiation-induced fibrosarcoma: a 31P NMR investigation. Proc Natl Acad Sci USA 1984;81:6496-6500.
22. Evanochko WT, Ng TC, Lilly MD et al. In vivo 31-P NMR study of the metabolism of murine mammary 16/C adenocarcinoma and its response to chemotherapy, x-radiation, and hyperthermia. Proc Natl Acad Sci USA 1983;80:334-338.
23. Melner MH, Sawyer ST, Evanochko WT et al. Phosphorus-31 nuclear magnetic resonance analysis of epidermal growth factor action in A-431 human epidermoid carcinoma cells and SV-40 virus transformed mouse fibroblasts. Biochemistry 1983;22:2039-2042.
24. Koutcher JA, Damadian R. Spectral differences in the 31P NMR of normal and malignant tissue. Physiological Chemistry & Physics 1977;9:181-187.
25. Negendank W. Studies of human tumors by MRS: a review. NMRiB 1992;5:303-324.

26. Negendank W. Studies of human tumors by MRS: A review. NMR in Biomedicine 1992;5:303-324.
27. Negendank WG, Brown TR, Evelhoch JL et al. Proceedings of a National Cancer Institute workshop: MR spectroscopy and tumor cell biology. Radiology 1992;185:875-883.
28. Griffiths JR. Are cancer cells acidic? Br J Cancer 1991;64:425-427.
29. Gillies RJ, Liu Z, Bhujwalla Z. 31P-MRS measurements of extracellular pH of tumors using 3-aminopropylphosphonate. American Journal of Physiology 1994;267:c195-c203.
30. Gil S, Zaderenzo P, Cruz F et al. Imidazol-1-ylalkanoic Acids as Extrinsic 1H NMR Probes for the Determination of Intracellular pH, Extracellular pH and Cell Volume. Bioorganic & Medicinal Chemistry Letters 1994;2:305-314.
31. Gil MS, Cruz F, Cerdan S et al. Imidazol-1-Ylalkanoate Esters And Their Corresponding Acids. A Novel Series of Extrinsic 1H NMR Probes For Intracellular pH. Bioorg Med Chem Lett 1992;2:1717-1722.
32. van Sluis R, Bhujwalla ZM, Raghunand N et al. In vivo imaging of extracellular pH using 1H MRSI. Magnetic Resonance in Medicine 1999;41:743-750.
33. Bhujwalla ZM, Artemov D, Ballesteros P et al. Combined vascular and extracellular pH imaging of solid tumors. NMR in Biomedicine 2002;15:114-9.
34. Garcia-Martin ML, Herigault G, Remy C et al. Mapping extracellular pH in rat brain gliomas in vivo by 1H magnetic resonance spectroscopic imaging: comparison with maps of metabolites. Cancer Res 2001;61:6524-6531.
35. Vermathen P, Capizzano AA, Maudsley AA. Administration and (1)H MRS detection of histidine in human brain: application to in vivo pH measurement. Magnetic Resonance in Medicine 2000; 43:665-675.
36. Deutsch C, Erecinska M, Werrlein R et al. Cellular energy metabolism, trans-plasma and trans-mitochondrial membrane potentials, and pH gradients in mouse neuroblastoma. Proc Natl Acad Sci USA 1979;76:2175-2179.
37. Taylor JS, Deutsch C. Fluorinated - methylamino acids as 19-F NMR indicators of intracellular pH. Biophysical Journal 1983;43:261-267.
38. Mason RP. Transmembrane pH gradients in vivo: measurements using fluorinated vitamin B6 derivatives. Current Medicinal Chemistry 1999;6:481-499.
39. Hunjan S, Mason RP, Mehta VD et al. Tumor transmembrane pH gradient measurement with 6-fluorophyridoxol by 19F NMR spectroscopy. Magnetic Resonance in Medicine 1998;39:551-556.
40. Ojugo AS, McSheehy PM, McIntyre DJ et al. Measurement of the extracellular pH of solid tumours in mice by magnetic resonance spectroscopy: a comparison of exogenous (19)F and (31)P probes. NMR in Biomedicine 1999;12:495-504.
41. Aoki Y, Akagi K, Tanaka Y et al. Measurement of intratumor pH by pH indicator used in 19F-magnetic resonance spectroscopy. Measurement of extracellular pH decrease caused by hyperthermia combined with hydralazine. Investigative Radiology 1996;31:680-689.
42. Mikawa M, Miwa N, Brautigam M et al. Gd(3+)-loaded polyion complex for pH depiction with magnetic resonance imaging. Journal of Biomedical Materials Research 2000;49:390-395.
43. Zhang S, Wu K, Sherry AD. A Novel pH-Sensitive MRI Contrast Agent. Angewandte Chemie Int. Ed. 1999;38:3192-3194.
44. Beauregard DA, Parker D, Brindle KM. Relaxation-based mapping of tumour pH. Proc. International Society for Magnetic Resonance in Medicine 1998;6:53.
45. Raghunand N, Howison C, Zhang S et al. Renal and Systemic pH Imaging by Contrast-Enhanced MRI. Magnetic Resonance in Medicine 2003;49:249-257.
46. Hwang TL, van Zijl PC, Mori S. Accurate quantitation of water-amide proton exchange rates using the phase-modulated CLEAN chemical EXchange (CLEANEX-PM) approach with a Fast-HSQC (FHSQC) detection scheme. Journal of Biomolecular NMR 1998;11:221-226.
47. Mori S, Eleff SM, Pilatus U et al. Proton NMR spectroscopy of solvent-saturable resonances: a new approach to study pH effects in situ. Magnetic Resonance in Medicine 1998;40:36-42.
48. Ward KM, Balaban RS. Determination of pH Using Water Protons and Chemical Exchange Dependant Saturation Transfer (CEST). Magnetic Resonance in Medicine 2000;44:799-802.
49. Ward KM, Aletras AH, Balaban RS. A new class of contrast agents for MRI based on proton chemical exchange dependent saturation transfer (CEST). J. Magn. Reson. 2000;143:79-87.
50. Aime S, Barge A, Castelli DD et al. Paramagnetic Lanthanide(III) complexes as pH-sensitive Chemical Exchange Saturation Transfer (CEST) contrast agents for MRI applications. Magnetic Resonance in Medicine 2002;47:639-648.

51. Zhang S, Winter P, Wu K et al. A novel europium(III)-based MRI contrast agent. Journal of the American Chemical Society 2001;123:1517-1518.
52. Zhang S, Sherry AD. Paramagnetic CEST agents: a comparison of NH vs. OH exchange. Proc International Society for Magnetic Resonance in Medicine 2002.
53. Hanigan MH, Gallagher BC, Townsend DM, Gabarra V. Gamma-glutamyl transpeptidase accelerates tumor growth and increases the resistance of tumors to cisplatin in vivo. Carcinogenesis 1999;20:553-559.
54. Matsumoto H, Inoue N, Takaoka H, Hata K, Shinke T, Yoshikawa R, Masai H, Watanabe S, Ozawa T, Yokoyama M. Depletion of antioxidants is associated with no-reflow phenomenon in acute myocardial infarction. Clin Cardiol 2004;27:466-470.
55. Kuppusamy P, Li H, Ilangovan G, Cardounel AJ, Zweier JL, Yamada K, Krishna MC, Mitchell JB. Noninvasive imaging of tumor redox status and its modification by tissue glutathione levels. Cancer Res 2002;62:307-312.
56. Gallez B, Gacic G, Goda F, Jiang J, O'Hara JA, Dunn JF, Swartz HM. Use of nitroxides for assessing perfusion, oxygenation, and viability of tissues: in vivo EPR and MRI studies. Magn Reson Med 1996;35:97-106.
57. Mikuni T, He G, Petryakov S, Fallouh MM, Deng Y, Ishihara R, Kuppusamy P, Tatsuta M, Zweier JL. In vivo detection of gastric cancer in rats by electron paramagnetic resonance imaging. Cancer Res 2004;64:6495-6502.
58. Jonas CR, Ziegler TR, Gu LH, Jones DP. Extracellular thiol/disulfide redox state affects proliferation rate in a human colon carcinoma (Caco2) cell line. Free Radical Biol Med 2002;33:1499-1506.
59. Pallis M, Bradshaw TD, Westwell AD, Grundy M, Stevens MFG, Russell N. Induction of apoptosis without redox catastrophe by thioredoxin-inhibitory compounds. Biochem Pharmacol 2003;66:1695-1705.
60. Jonas CR, Gu LH, Nkabyo YS, Mannery YO, Avissar NE, Sax HC, Jones DP, Ziegler TR. Glutamine and KGF each regulate extracellular thiol/disulfide redox and enhance proliferation in Caco-2 cells. Am J Physiol 2003;285:R1421-R1429.
61. Rehman F, Shanmugasundaram P, Schrey MP. Fenretinide stimulates redox-sensitive ceramide production in breast cancer cells: potential role in drug-induced cytotoxicity. Br J Cancer 2004;91:1821-1828.
62. Rowinsky EK. Novel radiation sensitizers targeting tissue hypoxia. Oncology 1999;13:61-70.
63. Brown JM, Wilson WR. Exploiting tumour hypoxia in cancer treatment. Nat Rev Cancer 2004;4:437-447.
64. Welsh SJ, Williams RR, Birmingham A, Newman DJ, Kirkpatrick DL, Powis G. The thioredoxin redox inhibitors 1-methylpropyl 2-imidazolyl disulfide and pleurotin inhibit hypoxia-induced factor 1alpha and vascular endothelial growth factor formation. Mol Cancer Therap 2003;2:235-243.
65. Aime S, Botta M, Gianolio E, Terreno E. A pO2-responsive MRI contrast agent based on the redox switch of Manganese (II/III)-porphyrin complexes. Angew Chem Intl Ed 2000;39:747-750.
66. Burai L, Toth E, Seibig S, Scopelliti R, Merbach AE. Solution and solid-state characterization of Eu(II) chelates: a possible route towards redox responsive MRI contrast agents. Chem Eur J 2000;6:3761-3770.